Review

Vladislav Dolník
Shaorong Liu
Stevan Jovanovich

Molecular Dynamics,
Sunnyvale, CA, USA

Capillary electrophoresis on microchip

Capillary electrophoresis and related techniques on microchips have made great strides in recent years. This review concentrates on progress in capillary zone electrophoresis, but also covers other capillary techniques such as isoelectric focusing, isotachophoresis, free flow electrophoresis, and micellar electrokinetic chromatography. The material and technologies used to prepare microchips, microchip designs, channel geometries, sample manipulation and derivatization, detection, and applications of capillary electrophoresis to microchips are discussed. The progress in separation of nucleic acids and proteins is particularly emphasized.

Keywords: Capillary electrophoresis / Microchip / Microdevices / Microfabrication / Micromachining / Review EL 3712

Contents

1 Introduction 41
2 Materials and fabrication technologies 42
2.1 Fabrication procedures for glass materials . 42
2.2 Fabrication procedures for polymer materials 42
2.2.1 Laser ablation 42
2.2.2 Injection molding, embossing and casting . . 43
2.2.3 Groove enclosure 43
3 Microchip format 43
3.1 Microchip layout 44
3.2 Separation channel length 45
3.3 Channel geometry 45
4 Sample manipulation and derivatization . . . 46
4.1 Sample injection and zone manipulation . . . 46
4.2 On-line sample derivatization 46
5 Detection 46
5.1 Optical detection 46
5.2 Mass spectrometry 47
5.3 Electrochemical detection 47
5.4 Other detection methods 47
6 Applications 47
6.1 Nucleic acids 47
6.1.1 Nucleic acid sizing 48
6.1.2 Genotyping 48
6.1.3 DNA sequencing 48
6.1.4 Integrated nucleic sample preparation and analysis 48
6.2 Peptides and proteins 49
6.3 Other applications 50
7 Conclusions and outlook 51
8 References 51

Correspondence: Dr. Vladislav Dolník, Molecular Dynamics, 928 East Arques Avenue, Sunnyvale, CA, 94086, USA
E-mail: vdolnik@mdyn.com
Fax: +408-737-4808

Abbreviation: CAE, capillary array electrophoresis

1 Introduction

Capillary electrophoresis on microchips is an emerging new technology that promises to lead the next revolution in chemical analysis. It has the potential to simultaneously assay hundreds of samples in a matter of minutes or less. The rapid analysis combined with massively parallel analysis arrays should yield ultrahigh throughput. Microchips typically consume only picoliters of samples. These samples may potentially be prepared on-board for a complete integration of sample preparation and analysis functions. These features make microchips an attractive technology for the next generation of capillary electrophoresis instrumentation.

The first instrument on a microchip was an integrated gas chromatograph [1]. While this device never commercially succeeded, it nonetheless initiated the application of micromachining technology to build chemical analysis devices. By the early 90s, chemical analysis on microchips had been demonstrated for many capillary electrophoresis applications [2–10]. Microfabricated devices have so far separated fluorescently labeled amino acids [11], DNA restriction fragments [4, 6, 12], PCR products, short oligonucleotides [13], and sequencing ladders (reviewed in [15–26]).

Capillary electrophoresis on microchips is based upon microfabrication techniques developed in the semiconductor industry. Microchannels are fabricated in microchips using photolithography or micromolding to form channels for sample injection and capillary electrophore-

CE and CEC

© WILEY-VCH Verlag GmbH, 69451 Weinheim, 2000
0173-0835/00/0101-0041 $17.50+.50/0

sis separation. After all solutions, including samples, are loaded, samples are typically transferred electrokinetically into an injector region. The samples are then separated by applying high voltage while a potential is applied to the sample and waste reservoirs to prevent the sample from bleeding [13]. The analytes are detected by laser-induced fluorescence (LIF) or other methods. The small injection plugs, high fields, and short separation lengths produce separation times measured in seconds or minutes. Because fairly standard microfabrication technology is used, mass production of microchip devices for capillary electrophoresis should be economically feasible. The following sections give an overview of fabrication methods, microchip designs, and applications of capillary electrophoresis to microchips.

2 Materials and fabrication technologies

CE chips are mainly fabricated using various glass substrates [6, 27–30], from inexpensive soda lime glass to high quality quartz. Glass substrates are the most common substrates because of their good optical properties, well-understood surface characteristics, and well-developed microfabrication methods, adapted from the microelectronics industry. Recently, various polymer materials have been used to fabricate microchips for CE separations [31–34]. Polymer microchips are of increasing interest because their potentially low manufacturing costs may allow them to be disposable. Fabrication procedures for these materials are quite different from those for glass. The following sections describe typcial fabrication procedures for both types of materials.

2.1 Fabrication procedures for glass materials

Structures on glass substrates are usually generated using standard photolithographic technologies [6, 27–29]. Figure 1 presents a diagram of such a procedure. First a sacrificial etching mask layer is attached to the surface to be structured. The most often used sacrificial mask is Cr/Au [5, 27, 29, 35]. The thin (usually 100–500 Å) film of Cr is used to enhance the adhesion between the substrate and the gold layer, which is the real sacrificial mask. The Cr/Au film is effective for almost all types of glass etchants, particularly hydrogen fluoride (HF)/HNO_3 which attacks other etch masks, such as amorphous silicon. An alternative mask is amorphous silicon [36, 37]. Amorphous silicon normally adheres to glass better than Cr/Au and works well for concentrated HF as an etchant. High quality and very deep channels (> 70 μm) can be etched with few defects [38]. A layer of photoresist can also be used as a mask to etch shallow channels [6, 12, 39, 40]. In this case, the photoresist serves two functions, a sacrificing mask and a regular photoresist, to transfer photomask patterns.

The pattern to be etched is then transferred to the wafer. First, a layer of photoresist is spin-coated on top of the mask layer. Photoresist is a polymer that becomes soluble (for positive photoresist) or insoluble (for negative photoresist) in developer solutions after exposure to light. In the next step, the photoresist spun on the microchip is exposed in the region defined by a photomask, typically using an aligner. The photomask is a plate with a user-designed pattern that is transparent while the background is opaque (or *vice versa*) to the exposition light. After the microchip is baked to harden unexposed resist, the exposed photoresist is dissolved with a developer solution. The sacrificial mask layer of the exposed region is removed using the appropriate etchants. During this time, the sacrificial layer underneath the unexposed photoresist remains intact.

After pattern transfer and development, the portions of the microchip that are to be etched have been unmasked and are now ready for chemical etching. HF is used as the primary etchant and can be prepared in various solutions including HF/NH_4F, HF/HNO_3, and concentrated HF. The etching rate of HF for glass is readily controllable if the temperature is controlled. The etching progress can be monitored with a profilometer. Following etching of the microchannels, the photoresist and sacrificing mask layer are stripped, and the access holes drilled (not shown in Fig. 1). The access holes can be drilled on the etched substrate or on another blank glass wafer. When holes are drilled on the etched substrate, aligning two substrates for bonding is much easier. Finally, the substrate is bonded to another piece of substrate to form a finished microchip. Thermal diffusion is the most often used method for glass bonding [3, 41]. Other methods such as chemical activated bonding [42] and adhesive annealing [43, 44] are also used.

2.2 Fabrication procedures for polymer materials

Methods for the fabrication of plastic microchips include laser ablation [31], injection molding [32, 45], silicone rubber casting [33], and hot embossing [34].

2.2.1 Laser ablation

The photoablation process involves absorption of a short-wavelength laser pulse to break covalent bonds in long-chain polymer molecules with production of a shock wave that ejects decomposed polymer fragments [46]. Many commercially available polymers can be photoablated,

including polycarbonate, poly(methyl methacrylate) (PMMA), polystyrene, nitrocellulose, poly(ethylene terephthalate) (PET or Melinex), and poly(tetrafluoroethylene) (Teflon) [31, 47]. The laser energy can be specially patterned using a mask with the subsequent generation of microcavities and channels in various geometries or by controlling position of the laser with *x-y* stages. The resulting structures are generally characterized as having little thermal damage, straight vertical walls, and well-defined depth [46, 48]. However, laser ablation does not lend itself well to mass production.

2.2.2 Injection molding, embossing and casting

The formation of microchannels and other structures using molding methods generally involves two primary steps: (i) fabrication of a molder (also known as a master), and (ii) channel pattern transfer from the molder to polymer substrates. Various techniques can be used to produce a molder depending upon the channel dimensions and precision requirements. For large structures (> 100 μm), traditional computer numerical control (CNC) machining of materials like stainless steel can be sufficiently accurate. For smaller features (< 100 μm), a silicon wafer or a thick photoresist structure is etched and then electroplated with a metallic material such as nickel or nickel-cobalt. For very small channels with high aspect ratios, lithography, electroplating and molding (LIGA), followed by electroplating [49] is the method of choice to produce the molder; LIGA is a process that uses a synchrotron to produce X-rays for photolithography of an X-ray resist, followed by electroplating to form a molder.

Replication of the molder to produce microchips can be accomplished by injection molding, embossing, or casting. In the injection molding process, polymer is melted and injected against the molder in the molding chamber. Molded devices are released every 5–10 s. Injection molding allows very high-throughput production with low production costs [32]. In the embossing process [50], the embossing tool and the polymer substrate are heated separately under vacuum (this extends the lifetime of the molder) to a temperature just above the glass transition temperature of the polymer material. The tool is then brought into contact with the substrate and embossed. Embossing can take several minutes per device and can be a useful tool in rapid prototyping devices. In the casting process, polymer material such as poly(dimethylsiloxane) (PDMA) is usually poured on the top of the molder and then cured/hardened [33] at atmospheric pressure and temperature. Elevating the temperature can accelerate the curing. Casting is the simplest among these three molding processes, but requires contact with the molder for minutes or hours.

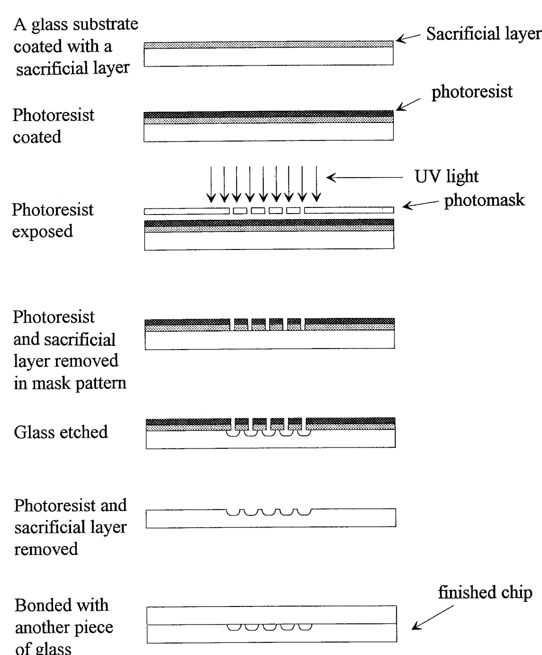

Figure 1. Photolithographic process for making chips.

2.2.3 Groove enclosure

Thermal lamination is normally used to seal grooves to form channels [31, 32]. Channels formed using this method can have two different surface types, three walls of the polymer substrate and one wall of the laminated film. If the materials are not well matched or appropriately post-modified, plug flow is disrupted due to the different characteristics of the two surfaces and the separation is degraded. Another bonding technique is to anneal the molded plate directly to another plate at room temperature [33, 51]. Very strong bonding (possibly some covalent bonding) was reported after two PDMA surfaces were treated with oxygen plasma [51]. Clean PDMA surfaces also strongly bonded to other surfaces such as glass and plastics [33]. When a PDMA-molded plate is bonded with a thin slab of PDMA, four equivalent walls are formed [33]. Channels with four equivalent walls can also be made with other polymeric materials [50]; however, details of the bonding procedure have not been described.

3 Microchip format

The design of microchips for capillary electrophoresis (CE) has undergone significant development from simple single-channel structures to increasingly complex ones. Design rules for many channel geometries and separation lengths are being developed and computer-aided design

tools are becoming commercially available. Current designs of CE chips allow reactions on-chip and separations in multiple channels.

3.1 Microchip layout

During the past several years, significant progress has been made in the design of CE chips (Fig. 2). The first CE chips used single microchannels fabricated on relatively large-scale substrates (14.8 × 3.9 cm) [3] (Fig. 2a). Several years later, miniaturized chips appeared containing two crossed channels and four reservoirs for sample, waste, and cathode and anode electrolytes [4]. Separation channel lengths can be increased by introducing serpentine turns into the separation channels (Fig. 2b) [5]. To further extend the separation path, a synchronized cyclic CE chip has been designed: the channel forms a

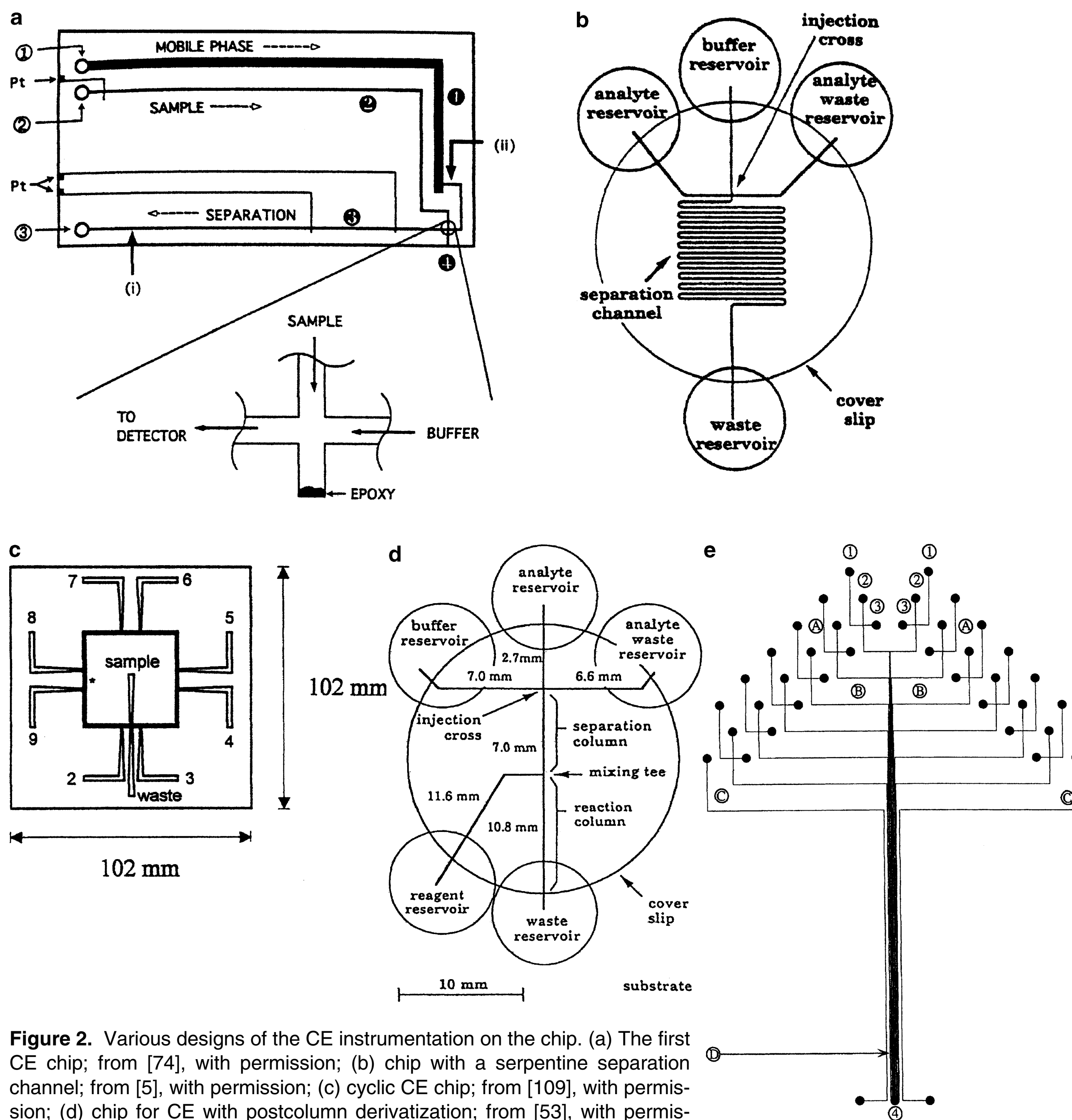

Figure 2. Various designs of the CE instrumentation on the chip. (a) The first CE chip; from [74], with permission; (b) chip with a serpentine separation channel; from [5], with permission; (c) cyclic CE chip; from [109], with permission; (d) chip for CE with postcolumn derivatization; from [53], with permission; (e) mask design of a chip for 12-channel capillary array electrophoresis; from [12], with permission.

loop with two pairs of electrodes connected. Switching voltage between pairs of electrodes enables separands to migrate in cycles around the loop and thus to increase the migration path (Fig. 2c) [52]. Microreactors can also be added to perform on-line reactions such as sample preparation (see Section 6.1) or postcolumn derivatization (see Section 4.2) (Fig. 2d) [53]. Some other chip layouts constructed include ones with a rectangular channel where the sample is loaded by a sampling capillary [54] and free-flow electrophoresis microchips [55]. These are just few examples; naturally more single-channel variants are in use.

To simultaneously analyze large number of samples, arrays of separation channels have been designed on microchips [12, 26, 36]. Multichannel microchips present unique design constraints. The geometry of multiple separation channels can be constrained by substrate size, requirements for straight channels, and by the detection method. Sample and waste wells can consume significant real estate and usually need to be laid out with geometries that match the loading device. Finally, all channels may be constrained to have similar geometries and equal distances from the cathode to the anode and from the injection region to the detector. One solution to many of these constraints is a multichannel microchip demonstrated for genotyping (Fig. 2e) [36]. This microchip uses 48 parallel separation channels to separate 96 samples in two sets of 48 samples in less than 8 min. A microchip with 96 radial separation channels has also been designed, as described in [15].

3.2 Separation channel length

The change of the CE column format from long capillaries to shorter microfabricated channels on microchips brings new opportunities, limitations, and challenges. Miniaturization, smaller injection plugs, and shorter separation paths enable rapid separations without significant peak broadening due to diffusion. If constant selectivity can be maintained, shorter separation paths can be used since resolution is proportional to the square root of the migration path when other contributors to peak broadening other than diffusion are ignored [56–59]. However, the shorter separation channels on microchips makes the length of the sample plug more critical. The contribution of the sample plug length to peak broadening can be calculated. When the conductivity of the sample zone equals that of the background electrolyte, the channel length L_{req} required for the full separation of separands *A* and *B* equals

$$L_{req} = L_{sample} \frac{\mu_B}{\mu_A - \mu_B}$$

where L_{sample} is the length of the sample plug and μ_A and μ_B are mobilities of the separands *A* and *B* [60]. To achieve separation in a separation channel of length L_{req}, the sample plug must not be longer than L_{sample}. The length of the sampling plug can be manipulated by electric field amplification [61–66], preconcentration by isotachophoresis [67–69], and, in micellar electrokinetic chromatography, by sweeping [70, 71]. In CE in sieving media, the interface between free electrolyte and polymer solution can also reduce the length requirements for the separation channel [72].

If the approaches described above are insufficient, a longer channel can simply be used alternatively. Microchips have been prepared with long straight channels of 20 cm and more [73]. Channel length can also be increased by using a serpentine geometry. An initial study showed that two rectangular corners in a 8.5 mm long channel did not increase zone dispersion significantly and it was predicted that a 50 cm long serpentine capillary channel could be fabricated in less than a 1 cm^2 area [74]. A real chip with a channel length of 165 mm has been produced in an area of less than 1 cm^2 [5]. Research examining zone dispersion, however, has shown that the impact of turns is more complex than previously thought [75, 76]. A zone of analyte migrating around the turn in a serpentine capillary is dispersed due to differences in the migration path at the inner and the outer perimeter of the turn. The amount of dispersion due to channel turns depends on the magnitude of the diffusion coefficient of the analyte. A one-dimensional model based on the ratio of the transverse analyte diffusion time to the analyte transit time around a turn allows prediction of the amount of the dispersion caused by turns [75].

3.3 Channel geometry

Microdevices for CE typically have channel depths of 15–40 μm and widths of 60–200 μm [4, 77, 78], although channels with depths of less than 10 μm have been reported [79, 80]. The small cross section of the separation channels and large thermal mass of the microchip allows Joule heat to be dissipated efficiently. Thus, high electric fields (over 2 kV/cm) can be applied on microchips [74]. An electric field as high as 53 kV/cm has been used in a specially designed channel to achieve sub-millisecond separations [80]. In free-air convection, small radial temperature gradients of 2–4°C from the center of the channel to the wall are formed. With forced cooling, the center-to-wall temperature difference is less than 1°C [37].

4 Sample manipulation and derivatization

Sampling, sample concentration by stacking, precolumn or postcolumn derivatization can be performed on-line in CE microchips. Samples are typically loaded from microtiter plates into sample wells using standard pipettors.

4.1 Sample injection and zone manipulation

Integrated sample injection is typically used to produce the small sample size (measured in picoliters) required for CE on microchips. The integrated injectors are usually either cross-channel injectors, formed by orthogonally intersecting the separation channel with a channel connecting the sample to waste, or twin-T injectors, where the two arms of the sample to waste channel are offset to form a larger injector region. Integrated sample injectors permit volume-defined electrokinetic sample injection [3, 5, 81, 82] of short injection plugs with reproducibility of peak heights better than 4.1% [83]. Band broadening of the plugs can occur from leakage at the injection channel intersection [27, 84]. Electrical biasing of the different reservoirs, such as in a pinched injection, minimizes the bleeding. When a modular injector on a microchip is connected to a fused separation capillary, the injection repeatability is also improved [85]. To enhance the detection limit, a stack injection can be applied to concentrate samples, as demonstrated for dansylated amino acids [86]. A major sample component can make CE analysis of trace sample components difficult or impossible. This type of interference can be significantly reduced or eliminated through zone manipulation [87]. Manipulation of zones during separation can be used to redirect them for fraction collection or other assays. Collection of zones by stopped-flow methods results in significant zone dilution. On microchips, selected zones can be redirected from the separation channel to a side channel electrically. The dilution of the isolated sample components hardly exceeded a factor of four [88].

4.2 On-line sample derivatization

To increase detection sensitivity of analytes without a strong inherent analytical signal, they are typically labeled with a fluorophore and detected by LIF. The labeling can be precolumn, *i.e.*, made prior to the CE separation, or postcolumn, when already separated analytes are labeled and detected. For precolumn sample derivatization, a 1 nL microreactor has been constructed [28]. Electrical control of background electrolyte, sample, and reagent stream allows a precise manipulation of the fluids within the channel manifold. Halftimes of the reaction between amino acids, arginine and lysine, and *o*-phthaldialdehyde are about 5 s with detection limits of 0.5–0.83 fmol [28].

For postcolumn sample derivatization, a microreactor was integrated after CE separation channels on a microchip [35, 53, 89]. The reactor geometry causes about 10% contribution to peak broadening. An on-chip postcolumn reaction of *o*-phthaldialdehyde and amino acids gives a separation efficiency of about 83 000 theoretical plates [35]. For chemiluminescence detection, a method of postcolumn labeling has been developed using horseradish peroxidase-catalyzed reaction of luminol with peroxide [82].

5 Detection

5.1 Optical detection

Sensitive detection schemes are essential in microfabricated devices for CE due to the extremely small size of the detection cell. LIF is so far the most popular detection scheme for CE chips because of its sensitivity [11, 28, 52, 53, 90–95]. A common LIF detection system that can be applied to microchips uses a confocal detection system, based on that described by Mathies and Huang [96]. A laser provides a coherent, collimated beam that is reflected by a dichroic beamsplitter into a high numerical aperture objective. The objective focuses the laser to a small beam waist inside the microchannel to excite fluorescently labeled analyte. The fluorescence emitted from fluorescently labeled analyte is collected and collimated by the objective, and passes back through the dichroic beamsplitter. In this direction, the beamsplitter reflects laser light and passes through the longer wavelength fluorescence light. An achromatic lens focuses the light onto the entrance of a spatial filter (confocal aperture) which is "confocal" with the microscope objective, *i.e.*, only light emitted from the focal region in the microchip passes through the pinhole; light scattered from the surface of the microchip and fluorescent light originating outside the channels are rejected. This increases the S/N ratio and can produce a sensitive detection system. The light exiting the spatial filter is directed to one or more detectors, such as photomultiplier tubes (PMTs) or a charge-coupled device (CCD) array. Filter sets and additional beamsplitters can be inserted to spectrally separate different emission channels. The output from the PMTs or CCD can be preamplified, digitized, and acquired by a computer for processing.

LIF detection of an array of microchannels in microchips presents additional requirements compared to capillaries or a single channel. The laser can be scanned over the microchannels, which reduces the duty cycle, or continuous illumination can be used, which reduces laser power density at each microchannel. Either method can reduce the S/N ratio. The quick transit of the samples past the

detector in microchips also requires higher sampling rates than capillary array electrophoresis (CAE). Development of a robust LIF detector for multichannel microchips remains a challenge, although it has been demonstrated for genotyping [36].

Absorption detectors can be used with electrophoresis chip applications, but detection sensitivity is a concern because of the limited optical path length of the microfabricated channel. With the development of stable low-current detection electronics, detection limits in the low micromolar range are readily achieved in conventional CE, where the optical path length ranges from 50 to 100 μm [97]. To further improve the detection sensitivity on microchips, a 140 μm U-shaped detection cell was fabricated that could detect 6 μM of fluorescein [92]. To incorporate this cell into a UV/Vis detector, two additional channels were fabricated on both side of the U-cell to host optical fibers to guide the source light to the cell and the transmitted light to a photodetector [92].

5.2 Mass spectrometry

Mass spectroscopy (MS) is a powerful tool in analytical chemistry. It performs the separation, detection, and identification of a broad range of compounds, including polypeptides and nucleic acids. MS has been successfully coupled to CE with various electrospray microdevices. CE on microchips and electrospray ionization (ESI)/MS have recently been connected [98–104]. These same devices are used to load samples into MS using electroosmotic flow without any CE separation. Multiple samples can be placed on the microdevice and analyzed in an automatic mode. Recently a pneumatic nebulizer has been introduced to generate a stable sample flow for electrospray, eliminating the need for an electrospray tip and allowing microfabrication of the device in a single etching step [105]. An integrated microfluidic system for MS analysis of proteins has been constructed where the sample flow and data analysis are completely under computer control [98–100]. However, voltage is used to pump the liquid sample by electroosmotic flow to the electrospray and MS without any electrophoretic separation.

5.3 Electrochemical detection

While LIF provides sensitivity even to the single molecule limit, the detection system is much larger than the microfabricated analysis devices. This reduces the benefits of miniaturization [106]. Electrochemical methods provide an alternative detection approach to address this issue. When electrodes are microfabricated, they generally result in higher sensitivity and quicker response times [8, 76, 107]. Gavin and Ewing [8, 107] made a microfabricated electrochemical array detector for continuous electrophoretic separations in narrow channels. An array of 100 platinum microelectrodes at 5 μm spacing (95 μm wide, 1.2–2 mm long, 0.2 μm high) was placed on the microchip at the exit of the narrow channels. This setup allows electrochemical detection of neurotransmitter separations [107]. An integrated electrochemical detector for DNA analysis on a microchip has been constructed [106]. An integrated electrochemical detector for DNA analysis on a microchip has been constructed [106] with a 10 μm wide working electrode fabricated 30 μm from the end of the separation channel. To minimize the interference of the high separation voltage, an off-column detection scheme is employed after the separation channel. A ΦX174 *Hae*III restriction digest is detected in an indirect detection mode with a detection limit of 28 zmol [106].

5.4 Other detection methods

Some other detection methods have been applied to CE chips including Raman spectroscopy and holographic refractive index detection. Raman spectroscopy has been demonstrated as a suitable detector for microchip [108] for the isotachophoretic separation of pesticides (paraquat and diquat). Raman spectra were generated with a 2 W, 532 nm $NdYVO_4$ laser and collected at 8 cm^{-1} resolution with the data acquisition rate of 2–5 spectra/s. The 980 cm^{-1} Raman band for the counter ion, sulfate, was used as an internal standard to correct for instrument variations. Working sample concentrations ranged from 10^{-5} to 10^{-7} M [108]. A holographic refractive index detector has been constructed for CE on microchips [109]. It was tested by analysis of model saccharides at a concentration of 33 mM. The detection limit is rather poor, but the method has potential as a universal detector.

6 Applications

A development of applications for a new technology is always a confirmation that the technology is maturing. Electrophoresis on microchips has now been applied to the analysis of nucleic acids, proteins, and other types of samples in research applications. Commercial applications are expected to appear soon.

6.1 Nucleic acids

One of the leading applications of CE on microchips is the analysis of nucleic acids. Microchips have analyzed oligonucleotides and RNA, and genotyped and sequenced DNA. The analyses are extremely rapid, from less than a minute for oligonucleotides [13] to less than 20 min for DNA sequencing [14]. DNA is typically detected with LIF, but electrochemical detection has been applied as well

[106]. Microchips are being developed for commercial applications such as genotyping medically important loci and sequencing genomic human DNA. In addition, efforts are being made to integrate sample preparation and CE analysis on microchips.

6.1.1 Nucleic acid sizing

Microchips have been applied to size short oligonucleotides (10–25 bases) [13] and restriction fragments such as ΦX174 *Hae*III DNA [6, 32, 33, 51, 110], and ribosomal RNA [111]. Separation of ΦX174 *Hae*III DNA fragments from 70 to 1000 bp is completed in 120 s using an array of microchannels [6].

6.1.2 Genotyping

Genotyping by microchip CE is a rapidly developing application allowing a quick identification of genes responsible for hereditary diseases, such as hemochromatosis and Duchenne/Becker muscular dystrophy, and eventually for pharmacogenetics. In 1997, CAE on microchips analyzed restriction fragment markers from the HLA-H gene, a candidate gene for the diagnosis of hereditary hemochromatosis. Twelve samples were analyzed in parallel with a LIF scanner and an intercalating dye, thiazole orange [12]. Recently, 96 hemochromatosis samples were analyzed in less than 8 min on a microchip with 96 sample reservoirs and 48 analysis capillaries using a LIF scanner [36]. Locus-specific, multiplex PCR products specific for deletions causing Duchenne/Becker muscular dystrophy have been separated on a silicon-glass microchip [112]. Single-channel CE microchips with a 2.6 cm separation length and a replaceable polyacrylamide matrix have been used to analyze fluorescently-labeled CTTv PCR samples (containing the four loci CSF1PO, TPOX, THO1, and vWA) and short tandem repeats [113]. This body of work firmly establishes the feasibility of using CE microchips for genotyping. Commerical high-speed, high-throughput genotyping applications are now under development.

6.1.3 DNA sequencing

DNA sequencing with CAE on microchips is an area of intense interest, particularly with the exciting developments in the Human Genome Program. DNA sequencing with microchips was first demonstrated in 1995 in the Mathies lab [39]. Glass microchips with a denaturing polyacrylamide sieving matrix were used to separate DNA sequencing fragment ladders fluorescently labeled with energy transfer dye primers. Single base resolution reached 150–200 bases in 10–15 min with an effective separation channel distance of 3.5 cm [39]. To achieve longer read-lengths, a microchip with long separation channels has been constructed [73]. Using a single-color detector, a DNA sequencing sample can be separated in less than 14 min with a 11.5 cm single-channel CE microchip using a linear polyacrylamide matrix. The resolution should be sufficient to achieve read-lengths of 400 bases [57]. An alternative approach to extend read-lengths on microchips is to use shorter channels and optimize the separation conditions. Using a four-color detector and a linear polyacrylamide matrix, read-lengths of over 500 bases were achieved in about 20 min in a single channel run at 99.4% accuracy (Fig. 3) [14]. This demonstrated the feasibility of high-speed, high-throughput four-color DNA sequencing using CE on microchips [14].

6.1.4 Integrated nucleic sample preparation and analysis

Integrating sample preparation and analysis is one of the prime goals of the micro-total-analysis system (μ-TAS) approach. Applications of μ-TAS to nucleic acid sample preparation and integrated analysis were first published in 1996 [40, 114–116]. One integrated device performs an automated restriction digest, and injects and separates the restriction fragments by CE [114]. Another reported developing components for thermocapillary pumping, thermal-cycling chambers, gel electrophoresis, and on-board DNA detection [115]. PCR reactors microfabricated in silicon have been coupled to CE microchips to create an integrated DNA analysis system [40, 116]. The microfabricated PCR device has rapid thermal cycling characteristics and *Salmonella* genomic DNA can be amplified and analyzed in under 45 min [40]. These reports demonstrated that high-speed DNA analyses could be integrated with sample preparation on microchips.

An integrated monolithic microchip was used recently for cell lysis, multiplex PCR amplification, and electrophoretic analysis [117]. PCR targets such as bacteriophage λ, *Escherichia coli* genomic DNA, and plasmid DNA can be amplified from *E. coli* cells on a microchip and then electrophoretically separated in less than 3 min [117]. A different approach performed individual sample preparation reactions on individual microchips and then analyzed them on a CE microchip [112]. In this study, random nonspecific amplifications of the human genome were performed on one microchip and then aliquots were loaded into another microchip for a locus-specific, multiplex PCR for Duchenne/Becker muscular dystrophy. The amplicons were then analyzed both by traditional CE and by CE on microchips, with similar results. This segmented approach demonstrated that complex sample preparation and analysis can be performed on microchips [112].

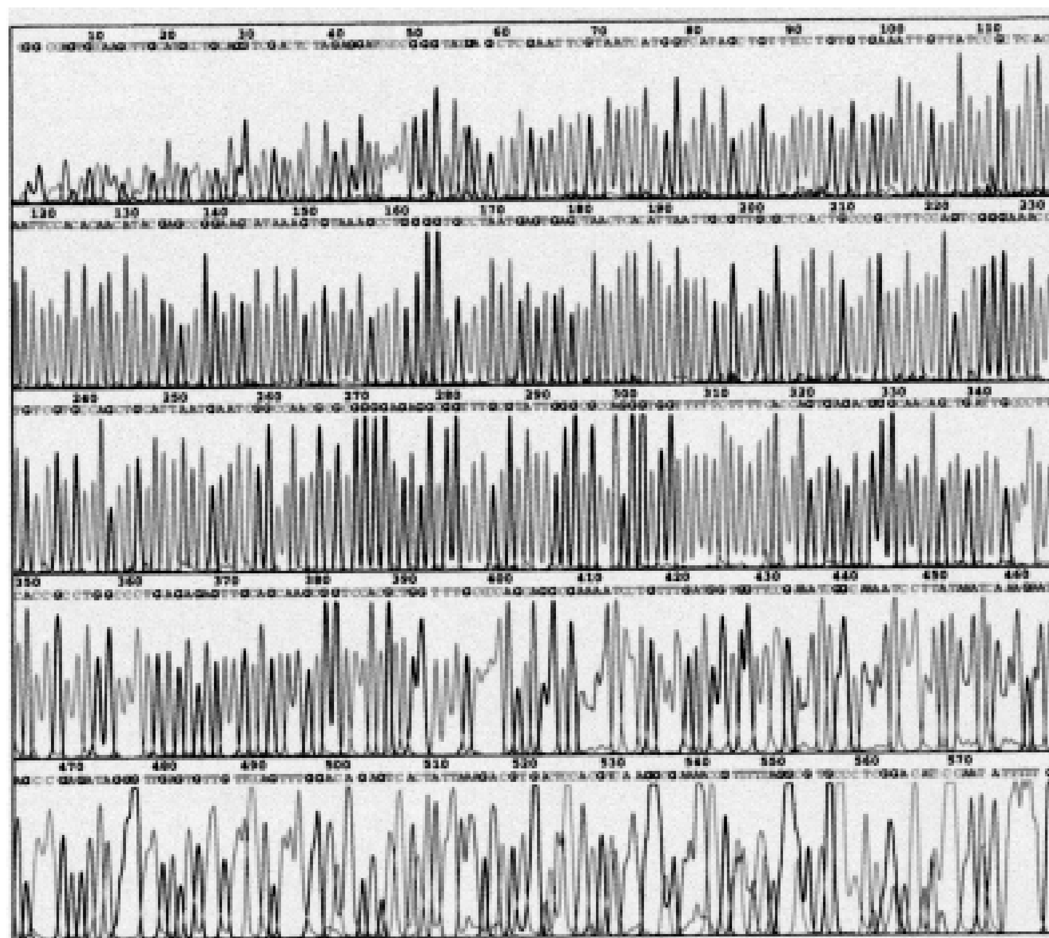

Figure 3. DNA sequencing by CE on microchip. The M13 sequencing trace has been processed and base-called with BaseFinder software [135]. The accuracy was 99.4% out to 500 bases. From [14], with permission.

Finally, an integrated silicon device has been microfabricated that prepares DNA samples, separates fragments, and detects samples with an on-board integrated photodiode [118]. This *tour de force* microchip is capable of measuring and moving samples in microchannels, with heaters, temperature sensors, and a fluorescence photodiode detector. Strand displacement amplification reaction was applied to amplify target DNA, mixed with intercalating dye, separated on cross-linked polyacrylamide gel, and detected with the photodiode, all on board the microchip. The appropriate fragment was detected in 4 min [118]. These results demonstrate the enormous potential for integration of sample preparation, analysis, and detection on CE microchips.

6.2 Peptides and proteins

Separation of proteins has always been an important part of the analysis of biological systems and more efforts have been devoted lately to separating proteins on microchips by CE. The rapid separation of proteins using zone electrophoresis, SDS electrophoresis, and isoelectric focusing have now been demonstrated. While the reports show the basic feasibility of protein separation, they are only a first glimmer of the potential of this powerful technology. Capillary SDS electrophoresis on a microchip has been reported recently. Fluorescein-labeled proteins (calmodulin, α-lactalbumin, pepsinogen, ovalbumin, BSA, and β-galactosidase) were separated in a 5 cm channel filled with SDS 24–200 (a sieving matrix from Beckman-Coulter) and detected by LIF detection. With an applied voltage from 1 to 5 kV, the separation time ranged from 35 to 200 s. Under optimized conditions, plate heights of *ca.* 1 μm can be obtained, whereas plate heights with the same proteins in capillary exceed 10 μm [119].

Two microchip devices for isoelectric focusing of proteins have recently been published [120, 121]. Capillary IEF

with detection on-line requires zone mobilization, bringing protein zones into the detector. The three most common methods of mobilization, chemical mobilization, pressure mobilization, and electroosmotic mobilization, of separated proteins were compared. It was found that electroosmotically driven mobilization, which occurs simultaneously with the focusing, is the most suitable technique for miniaturization because of its high speed, compatibility of electroosmotic flow, and minimal instrumentation requirements [120]. Further, Cy5-labeled peptides can be focused in less than 30 s in a 7 cm × 200 μm × 10 μm channel with a total analysis time of less than 5 min. The maximum peak capacity is about 30–40 peaks [120]. The other approach used UV imaging detection that eliminates the requirement of zone mobilization. The separation channel is 4 cm × 100 μm × 10 μm. The detection limit is 2.4 ng for myoglobin at the optical path length of 10 μm [121].

Capillary zone electrophoresis (CZE) has been applied to analyze several proteins. A mixture of model human proteins (albumin, α_1-antitrypsin, transferrin, and IgG), simulating the electrophoretic pattern of human serum proteins, has been separated by CZE on a microchip after labeling with 2-toluidinonaphthalene-6-sulfonate. Real-world samples of human serum proteins, however, did not provide the expected set of five traditional peaks due to the poor labeling of several serum proteins [122, 123]. Further, CE of antibodies was performed on microchips. Anti-human IgG labeled with fluorescein isothiocyanate (FITC) has been analyzed on a microchip with an effective length of 2.8 cm in less than 16 s [124]. Monoclonal mouse anti-BSA IgG was measured in mouse ascites fluid by a direct microchip-based CE immunoassay [125, 126]. The calibration curve is linear up to at least 135 mg/L. The method can measure the antigen-antibody interactions between BSA and anti-BSA and calculate stoichiometry and equilibration constants. A free-flow electrophoretic system has been constructed for continuous separation and micropreparation of proteins on microchips [55, 94]. The instrument was tested using BSA, bradykinin, and ribonuclease A as a model mixture. It can separate proteins and collect individual fractions for further use as shown on rat plasma [55, 94].

6.3 Other applications

An effort has been devoted to using CE microchips for the analysis of other types of compounds in addition to nucleic acids and polypeptides. Immunoassays, enzyme assays, micellar electrokinetic chromatography (MEKC) and isotachophoresis have been adapted to microchips and applied to assay herbicides, amino acids, biogenic amines, ions, and drugs.

Immunoassays followed by CE separations have been developed to analyze low molecular weight compounds in capillaries and in microchips (see review [127]). A competition immunoassay has been developed to determine the concentration of theophylline, a drug for the treatment of asthma, in serum samples [125, 128]. A sample containing unlabeled theophylline is mixed with known amounts of fluorescently labeled theophylline and theophylline antibody. The unlabeled theophylline molecules in the sample compete with the labeled molecules for the limited amount of antibody. As the content of theophylline in the sample increases, less labeled theophylline is bound to the antibody, resulting in a decreased signal in the theophylline-antibody complex and an increased signal for the free labeled theophylline. CE separation on microchips with LIF detection can separate and quantify the free theophylline and theophylline-antibody complex peaks. The limit of detection for theophylline in diluted serum is 1.25 μg/L with a separation time of less than 50 s [125, 128]. Immunoassays followed by CE on microchips have measured serum thyroxin [129] and serum cortisol [29]. For thyroxin, using fluorescein-labeled thyroxin and a polyclonal antibody preparation as assay reagents, serum thyroxin could be electrophoretically analyzed in about 15 s [129]. Serum cortisol can be measured with a microchip-based electrophoretic immunoassay over a range of clinical interest (10–600 μg/L) without sample preconcentration [29].

MEKC on microchips has been applied to analyze biogenic amines (putrescine, cadaverine, spermidine, spermine, histamine, tyramine, tryptamine, and phenylethylamine) after derivatization with FITC. The analysis time is about 80 s, the run-to-run reproducibility of migration times varies between 0.15 and 0.54%, day-to-day reproducibility of migration times is between 0.40 and 0.80%, and the detection limit varies between 2.94 μM (putrescine) and 6.57 μM (spermine). The method was applied to real samples by analyzing biogenic amines in soy sauce [130].

Amphetamine and its analogs, methamphetamine, 3,4-methylenedioxymethamphetamine, and β-phenylethylamine, were analyzed in human urine after FITC derivatization by CZE and MEKC in synchronized cyclic mode (see Section 3.2) [131]. Using solid-phase extraction to concentrate the urine, the limit of identification is about 10 mg/L, a value that is currently too high for practical applications. The synchronized cyclic mode of MEKC has also been applied to separate a mixture of FITC-labeled amino acids [132].

Isotachophoresis on a microchip has been combined with Raman spectroscopy detection to assay the herbicides

paraquat and diquat. Working sample concentrations ranged from 10^{-5} to 10^{-7} M [108]. Magnesium and calcium can be determined by electrophoresis on chip after sample stacking and on-chip complexation. After a gated injection, the sample is concentrated in the stacking channel and derivatized on-chip with 8-hydroxyquinolin-5-sulfonic acid. The limit of detection is 18 ppb (0.45 μM) for calcium and 0.5 ppb (21 nM) for magnesium [133].

7 Conclusions and outlook

CE on microchips is a rapidly emerging, new analytical technology. The fundamental feasibility of applying microfabrication to CE is now well established and numerous applications have been demonstrated. The adaptation of CE to microchips has many advantages. Integrated injectors produce well-defined sample plugs that can be resolved in short path lengths. The short plugs, good dissipation of Joule heating, and high field strengths result in extremely rapid separations that consume only picoliter sample volumes [13]. Microchips with both sample preparation and separation functionalities [117] raise the possibility of integrating sample preparation and analysis on a single device. This integration could allow matching the sample preparation and analysis requirements with only picoliter volumes of samples being prepared. Photolithographic micromachining methods make the task of producing arrays of structures on microchips as simple as altering computer-aided design (CAD) drawings and should make manufacturing less expensive than traditional CAE. The applications described above illustrate that these advantages of microchips can potentially be translated into ultrafast computer-controlled CE separations for a wide range of analytes.

To achieve this potential, microchip technology must overcome many challenges. The interface between the microworld of the chip and the outside world needs to be improved with novel hardware solutions and interconnections amenable to microfluidic sample handling and eventual automation. Microchips need to be integrated in a systems level solution to sample preparation and analysis, and smaller sample volumes prepared [134] if the potential savings of volumetric reduction are to be realized. This exciting technology will need to be hardened until it is ready for commercial applications with more robust methods and instrumentation developed that require less human intervention. Microchips must be developed that can either be reused, for glass microchips, or be disposable, for plastic microchips. If these and other challenges can be overcome, CE on microchips will become a major analytical technology.

The rapid progress that CE on microchips has made in the last decade suggests the impact it may have in the next decade. CE on microchips has the potential to be the first widespread analytical application of fundamentally new microfluidic and microchip technologies. Microchip-based CE instruments are expected to be developed that will automatically perform rapid electrophoresis applications with extremely high throughputs and minimal sample preparation costs. In the coming years, microchip-based technology may revolutionize many industrial CE applications including drug screening, single nucleotide polymorphism (SNP) analysis, resequencing for pharmacogenetics, proteomics, and diagnostics.

The authors gratefully acknowledge funding from the National Institute of Standards and Technology (Grant number 70NANB5H0131) and the National Institutes of Health (grant numbers 5 R01HG01775-03, 1 R43HG02980-01, and 2 R44HG01563-02).

Received July 29, 1999

8 References

[1] Terry, S. C., Jerman, J. H., Angell, J. B., *IEEE Trans Electron Devices* 1979, *26*, 880–886.

[2] Manz, A., Graber, N., Widmer, H. M., *Sens. Actuators B* 1990, *1*, 244–248.

[3] Harrison, D. J., Manz, A., Fan, Z. H.. Ludi, H., Widmer, H. M., *Anal. Chem.* 1992, *64*, 1926–1932.

[4] Jacobson, S. C., Hergenroder, R., Koutny, L. B., Ramsey, J. M., *Anal. Chem.* 1994, *66*, 1114–1118.

[5] Jacobson, S. C., Hergenroder, R., Koutny, L. B., Warmack, R. J., Ramsey, J. M., *Anal. Chem.* 1994, *66*, 1107–1113.

[6] Woolley, A. T., Mathies, R. A., *Proc. Natl. Acad. Sci. USA* 1994, *91*, 11348–11352.

[7] He, B., Regnier, F., *J. Pharm. Biomed. Anal.* 1998, *17*, 925–932.

[8] Gavin, P. F., Ewing, A. G., *J. Am. Chem. Soc.* 1996, *118*, 8932–8936.

[9] Cheng, J., Shoffner, M. A., Hvichia, G. E., Kricka, L. J., Wilding, P., *Nucleic Acids Res.* 1996, *24*, 380–385.

[10] Cheng, J., Shoffner, M. A., Mitchelson, K. R., Kricka, L. J., Wilding, P., *J. Chromatogr. A* 1996, *732*, 151–158.

[11] Harrison, D. J., Fan, Z. H., Seiler, K., Manz, A., Widmer, H. M., *Anal. Chim. Acta* 1993, *283*, 361–366.

[12] Woolley, A. T., Sensabaugh, G. F., Mathies, R. A., *Anal. Chem.* 1997, *69*, 2181–2186.

[13] Effenhauser, C. S., Paulus, A., Manz, A., Widmer, H. M., *Anal. Chem.* 1994, *66*, 2949–2953.

[14] Liu, S. R., Shi, Y. N., Ja, W. W., Mathies, R. A., *Anal. Chem.* 1999, *71*, 566–573.

[15] Freemantle, M., *C&EN* 1999, *77*, 27–36.

[16] Regnier, F. E., He, B., Lin, S., Busse, J., *Trends Biotech.* 1999, *17*, 101–106.

[17] Campana, A. M. G., Baeyens, W. R. G., Aboulenein, H. Y., Zhang, X. R., *J. Microcol. Sep.* 1998, *10*, 339–355.

[18] Mastrangelo, C. H., Burns, M. A., Burke, D. T., *Proc. IEEE* 1998, *86*, 1769–1787.

[19] Mitchelson, K. R., Cheng, J., Kricka, L. J., *Trends Biotech.* 1997, *15*, 448–458.

[20] Effenhauser, C. S., Bruin, G. J. M., Paulus, A., *Electrophoresis* 1997, *18*, 2203–2213.

[21] Kopp, M. U., Crabtree, H. J., Manz, A., *Curr. Opinion Chem. Biol.* 1997, *1*, 410–419.

[22] Jacobson, S. C., Ramsey, J. M., in: Landers, J. P. (Ed.), *Handbook of Capillary Electrophoresis*, 2nd Ed., CRC Press, Boca Raton, FL 1997, pp. 827–839.

[23] Effenhauser, C. S., *Topics Curr. Chem.* 1998, *194*, 51–82.

[24] Kricka, L. J., *Clin. Chem.* 1998, *44*, 2008–2014.

[25] MacTaylor, C. E., Ewing, A. G., *Electrophoresis* 1997, *18*, 2279–2290.

[26] Paulus, A., *Amer. Lab.* 1998, *30*, No. 8, 59–62.

[27] Fan, Z. H., Harrison, D. J., *Anal. Chem.* 1994, *66*, 177–184.

[28] Jacobson, S. C., Hergenroder, R., Moore, A. W., Ramsey, J. M., *Anal. Chem.* 1994, *66*, 4127–4132.

[29] Koutny, L. B., Schmalzing, D., Taylor, T. A., Fuchs, M., *Anal. Chem.* 1996, *68*, 18–22.

[30] Jacobson, S. C., Moore, A. W., Ramsey, J. M., *Anal. Chem.* 1995, *67*, 2059–2063.

[31] Roberts, M. A., Rossier, J. S., Bercier, P., Girault, H., *Anal. Chem.* 1997, *69*, 2035–2042.

[32] McCormick, R. M., Nelson, R. J., Alonso-Amigo, M. G., Benvegnu, J., Hoopwer, H. H., *Anal. Chem.* 1997, *69*, 2626–2630.

[33] Effenhauser, C. S., Bruin, G. J. M., Paulus, A., Ehrat, M., *Anal. Chem.* 1997, *69*, 3451–3457.

[34] Martynova, L., Locascio, L. E., Gaitan, M., Kramer, G. W., Christensen, R. G., MacCrehan, W. A., *Anal. Chem.* 1997, *69*, 4783–4789.

[35] Fluri, K., Fitzpatrick, G., Chiem, N., Harrison, D. J., *Anal. Chem.* 1996, *68*, 4285–4290.

[36] Simpson, P. C., Roach, D., Woolley, A. T., Thorsen, T., Johnston, R., Sensabaugh, G. F., Mathies, R. A., *Proc. Natl. Acad. Sci. USA* 1998, *95*, 2256–2261.

[37] Liu, K. L. K., Davis, K. L., Morris, M. D., *Anal. Chem.* 1994, *66*, 3744–3750.

[38] Simpson, P. C., Woolley, A. T., Mathies, R. A., *Biomed. Microdevices* 1998, *1*, 7–26.

[39] Woolley, A. T., Mathies, R. A., *Anal. Chem.* 1995, *67*, 3676–3680.

[40] Woolley, A. T., Hadley, D., Landre, P., Demello, A. J., Mathies, R. A., Northrup, M. A., *Anal. Chem.* 1996, *68*, 4081–4086.

[41] Manz, A., Harrison, D. J., Verpoorte, E., Fettinger, J. C., Ludi, H., Widmer, H. M., *J. Chromatogr.* 1992, *593*, 253–258.

[42] Nakanishi, H., Abe, H., Nishimoto, T., Arai, A., *Bunseki Kagaku* 1998, *47*, 361–368.

[43] Wang, H. Y., Foote, R. S., Jacobson, S. C., Schneibel, J. H., Ramsey, J. M., *Sens. Actuators B* 1997, *45*, 199–207.

[44] Ewing, A. G., Gavin, P. F., Hietpas, P. B., Bullard, K. M., *Nature Med.* 1997, *3*, 97–99.

[45] Ekstrom, B., Jacobson, G., Ohman, O., Sjodin, H., *US Patent 5,376,252*, 1994.

[46] Reyna, L. G., Sobehart, J. R., *J. Appl. Phys.* 1994, *76*, 4367–4371.

[47] Srinivasan, R., Braren, B., *Chem. Rev.* 1989, *89*, 1303–1316.

[48] Niino, H., Yabe, A., *Appl. Surf. Sci.* 1993, *69*, 1–6.

[49] Ehrfeld, W., Munchmeyer, D., *Nucleic Instrum. Methods A* 1991, *303*, 523–532.

[50] Becker, H., Dietz, W., Dannberg, P., in: Harrison, D. J., Van den Berg, A. (Eds.), *Micro Total Analysis Systems '98*, Kluwer Academic Publishers, Boston, MA 1998, pp. 253–356.

[51] Duffy, D. C., McDonald, J. C., Schueller, O. J. A., Whitesides, G. M., *Anal. Chem.* 1998, *70*, 4974–4984.

[52] Burggraf, N., Manz, A., Effenhauser, C. S., Verpoorte, E., Derooij, N. F., Widmer, H. M., *J. High Res. Chromatogr.* 1993, *16*, 594–596.

[53] Jacobson, S. C., Koutny, L. B., Hergenroder, R., Moore, A. W., Ramsey, J. M., *Anal. Chem.* 1994, *66*, 3472–3476.

[54] Hietpas, P. B., Bullard, K. M., Gutman, D. A., Ewing, A. G., *Anal. Chem.* 1997, *69*, 2292–2298.

[55] Raymond, D. E., Manz, A., Widmer, H. M., *Anal. Chem.* 1994, *66*, 2858–2865.

[56] Luckey, J. A., Norris, T. B., Smith, L. M., *J. Phys. Chem.* 1993, *97*, 3067–3075.

[57] Schmalzing, D., Adourian, A., Koutny, L., Ziaugra, L., Matsudaira, P., Ehrlich, D., *Anal. Chem.* 1998, *70*, 2303–2310.

[58] Dolník, V., *Electrophoresis* 1997, *18*, 2353–2361.

[59] Kim, Y., Yeung, E. S., *J. Chromatogr. A* 1997, *781*, 315–325.

[60] Martin, A. J. P., Everaerts, F., *Proc. Roy. Soc. London* 1970, *316*, 493–514.

[61] Burgi, D. S., *Anal. Chem.* 1993, *65*, 3726–3729.

[62] Burgi, D. S., Chien, R. L., *Anal. Biochem.* 1992, *202*, 306–309.

[63] Burgi, D. S., Chien, R. L., *Anal. Chem.* 1991, *63*, 2042–2047.

[64] Burgi, D. S., Salomon, K., Chien, R. L., *J. Liq. Chromatogr.* 1991, *14*, 847–867.

[65] Chien, R. L., Burgi, D. S., *J. Chromatogr.* 1991, *559*, 141–152.

[66] Chien,R. L., Burgi, D. S., *J. Chromatogr.* 1991, *559*, 153–161.

[67] Dolník, V., Cobb, K. A., Novotny, M., *J. Microcol. Sep.* 1990, *2*, 127–131.

[68] Kanansky, D., Marák, J., *J. Chromatogr.* 1990, *498*, 191–204.

[69] Foret, F., Šustáček, V., Boček, P., *J. Microcol. Sep.* 1990, *2*, 229–233.

[70] Quirino, J. P., Terabe, S., *Anal. Chem.* 1999, *71*, 1638–1644.

[71] Quirino, J. P., Terabe, S., *Science* 1998, *282*, 465–468.

[72] Klepárník, K., Malá, Z., Boček, P., *J. Chromatogr. A* 1997, *772*, 243–253.

[73] Davidson, C., Balch, J., Brewer, L., Kimbrough, J., Swierkowski, S., Nelson, D., Madabhushi, R., Pastrone, R., Lee, A., McCready, P., Adamson, A., Bruce, R., Mariella, R.,

Carrano, A., *DOE Human Genome Program Contractor-Grantee Workshop VI*, Santa Fe, NM 1997.

[74] Seiler, K., Harrison, D. J., Manz, A., *Anal. Chem.* 1993, *65*, 1481–1488.

[75] Culbertson, C. T., Jacobson, S. C., Ramsey, J. M., *Anal. Chem.* 1998, *70*, 3781–3789.

[76] Gavin, P. F., Ewing, A. G., *J. Microcol. Sep.* 1998, *10*, 357–364.

[77] Jacobson, S. C., Hergenroder, R., Koutny, L. B., Ramsey, J. M., *Anal. Chem.* 1994, *66*, 2369–2373.

[78] Liu, Y. M., Sweedler, J. V., *Anal. Chem.* 1996, *68*, 2471–2476.

[79] Harrison, D. J., Fluri, K., Seiler, K., Fan, Z. H., Effenhauser, C. S., Manz, A., *Science* 1993, *261*, 895–897.

[80] Jacobson, S. C., Culbertson, C. T., Daler, J. E., Ramsey, J. M., *Anal. chem.* 1998, *70*, 3476–3480.

[81] Effenhauser, C. S., Manz, A., Widmer, H. M., *Anal. Chem.* 1993, *65*, 2637–2642.

[82] Mangru, S. D., Harrison, D. J., *Electrophoresis* 1998, *19*, 2301–2307.

[83] Evans, C. E., *Anal. Chem.* 1997, *69*, 2952–2954.

[84] Seiler, K., Fan, Z. H. H., Fluri, K., Harrison, D. J., *Anal. Chem.* 1994, *66*, 3485–3491.

[85] vanderMoolen, J. N., Poppe, H., Smit, H. C., *Anal. Chem.* 1997, *69*, 4220–4225.

[86] Jacobson, S. C., Ramsey, J. M., *Electrophoresis* 1995, *16*, 481–486.

[87] Dolník, V., Deml, M., Boček, P., in: Holloway, C. J. (Ed.), *Analytical And Preparative Isotachophoresis*, Walter de Gruyter, Berlin 1984, pp. 55–62.

[88] Effenhauser, C. S., Manz, A., Widmer, H. M., *Anal. Chem.* 1995, *67*, 2284–2287.

[89] Harrison, D. J., Fluri, K., Chiem, N., Tang, T., Fan, Z. H., *Sens. Actuators B* 1996, *33*, 105–109.

[90] Fister, J. C., Jacobson, S. C., Davis, L. M., Ramsey, J. M., *Anal. Chem.* 1998, *70*, 431–437.

[91] Flanagan, J. H., Owens, C. V., Romero, S. E., Waddell, L., Kahn, S. H., Hammer, R. P., Soper, S. A., *Anal. Chem.* 1998, *70*, 2676–2684.

[92] Liang, Z. H., Chiem, N., Ocvirk, G., Tang, T., Fluri, K., Harrison, D. J., *Anal. Chem.* 1996, *68*, 1040–1046.

[93] Ocvirk, G., Tang, T., Harrison, D. J., *Analyst* 1998, *123*, 1429–1434.

[94] Raymond, D. E., Manz, A., Widmer, H. M., *Anal. Chem.* 1996, *68*, 2515–2522.

[95] Zhang, Y., Lee, H. K., Li, S. F. Y., *Talanta* 1998, *45*, 613–618.

[96] Mathies, R. A., Huang, X. C., *Nature* 1992, *359*, 167–169.

[97] Bruin, G. J. M., Stegman, G., van Asten, A. C., Xu, X., Kraak, J. C., Poppe, H., *J. Chromatogr.* 1991, *559*, 163–181.

[98] Figeys, D., Aebersold, R., *J. Biochech. Eng. Trans. ASME* 1999, *121*, 7–12.

[99] Figeys, D., Aebersold, R., *Anal. Chem.* 1998, *70*, 3721–3727.

[100] Figeys, D., Ning, Y. B., Aebersold, R., *Anal. Chem.* 1997, *69*, 3153–3160.

[101] Ramsey, R. S., Ramsey, J. M., *Anal. Chem.* 1997, *69*, 1174–1178.

[102] Xu, N. X., Lin, Y. H., Hofstadler, S. A., Matson, D., Call, C. J., Smith, R. D., *Anal. Chem.* 1998, *70*, 3553–3556.

[103] Xue, Q. F., Foret, F., Dunayevskiy, Y. M., Zavracky, P. M., Mcgruer, N. E., Karger, B. L., *Anal. Chem.* 1997, *69*, 426–430.

[104] Xue, Q. F., Dunayevskiy, Y. M., Foret, F., Karger, B. L., *Rapid Commun. Mass Spectrom.* 1997, *11*, 1253–1256.

[105] Foret, F., Liu, H., Zhang, B., Felten, C., Karger, B. L., in: Harrison, D. J., Van Den Berg, A. (Eds.), *Micro Total Analysis Systems '98*, Kluwer Academic Publishers, Boston, MA 1998, pp. 35–38.

[106] Woolley, A. T., Lao, K. Q., Glazer, A. N., Mathies, R. A., *Anal. Chem.* 1998, *70*, 684–688.

[107] Gavin, . F., Ewing, A. G., *Anal. Chem.* 1997, *69*, 3838–3845.

[108] Walker, P. A., Morris, M. D., Burns, M. A., Johnson, B. N., *Anal. Chem.* 1998, *70*, 3766–3769.

[109] Burggraf, N., Krattiger, B., deMello, A. J., deRooij, N. F., Manz, A., *Analyst* 1998, *123*, 1443–1447.

[110] Effenhauser, C. S., Bruin, G. J. M., Paulus, A., *Electrophoresis* 1997, *18*, 2203–2213.

[111] Ogura, M., Agata, Y., Watanabe, K., McCormick, R. M., Hamaguchi, Y., Aso, Y., Mitsuhashi, M., *Clin. Chem.* 1998, *44*, 2249–2255.

[112] Cheng, J., Waters, L. C., Fortina, P., Hvichia, G., Jacobson, S. C., Ramsey, J. M., Kricka, L. J., Wilding, P., *Anal. Biochem.* 1998, *257*, 101–106.

[113] Schmalzing, D., Koutny, L., Adourian, A., Belgrader, P., Matsudaira, P., Ehrlich, D., *Proc. Natl. Acad. Sci. USA* 1997, *94*, 10273–10278.

[114] Jacobson, S. C., Ramsey, J. M., *Anal. cCem.* 1996, *68*, 720–723.

[115] Burns, M. A., Mastrangelo, C. H., Sammarco, T. S., Man, F. P., Webster, J. R., Johnson, B. N., Foerster, B., Jones, D., Fields, Y., Kaiser, A. R., Burke, D. T., *Proc. Natl. Acad. Sci. USA* 1996, *93*, 5556–5561.

[116] Woolley, A. T., Northrup, M. A., Mathies, R. A., *Abst. Paper Amer. Chem. Soc.* 1996, *212*, 155.

[117] Waters, L. C., Jacobson, S. C., Kroutchinina, N., Khandurina, J., Foote, R. S., Ramsey, J. M., *Anal. Chem.* 1998, *70*, 158–162.

[118] Burns, M. A., Johnson, B. N., Brahmasandra, S. N., Handique, K., Webster, J. R., Krishnan, M., Sammarco, T. S., Man, P. M., Jones, D., Heldsinger, D., Mastrangelo, C. H., Burke, D. T., *Science* 1998, *282*, 484–487.

[119] Yao, S., Anex, D. S., Caldwell, W. B., Arnold, D. W., Smith, K. B., Schultz, P. G., *Proc. Natl. Acad. Sci. USA* 1999, *96*, 5372–5377.

[120] Hofmann, O., Che, D. P., Cruickshank, K. A., Muller, U. R., *Anal. Chem.* 1999, *71*, 678–686.

[121] Mao, Q., Pawliszyn, J., *Analyst* 1999, *124*, 637–641.

[122] Colyer, C. L., Mangru, S. D., Harrison, D. J., *J. Chromatogr. A* 1997, *781*, 271–276.

[123] Colyer, C. L., Tang, T., Chiem, N., Harrison, D. J., *Electrophoresis* 1997, *18*, 1733–1741.

[124] Rodriguez, I., Zhang, Y., Lee, H. K., Li, S. F. Y., *J. Chromatogr. A* 1997, *781*, 287–293.

[125] Chiem, N., Harrison, D. J., *Anal. Chem.* 1997, *69*, 373–378.

[126] Chiem, N. H., Harrison, D. J., *Electrophoresis* 1998, *19*, 3040–3044.

[127] Schmalzing, D., Nashabeh, W., *Electrophoresis* 1997, *18*, 2184–2193.

[128] Chiem, N. H., Harrison, D. J., *Clin. Chem.* 1998, *44*, 591–598.

[129] Schmalzing, D., Koutny, L. B., Taylor, T. A., Nashabeh, W., Fuchs, M., *J. Chromatogr. B* 1997, *697*, 175–180.

[130] Rodriguez, I., Lee, H. K., Li, S. F. Y., *Electrophoresis* 1999, *20*, 118–126.

[131] Ramseier, A., vonHeeren, F., Thormann, W., *Electrophoresis* 1998, *19*, 2967–2975.

[132] vonHeeren, F., Verpoorte, E., Manz, A., Thormann, W., *Anal. Chem.* 1996, *68*, 2044–2053.

[133] Kutter, J. P., Ramsey, R. S., Jacobson, S. C., Ramsey, J. M., *J. Microcol. Sep.* 1998, *10*, 313–319.

[134] Kricka, L. J., *Clin. Chem.* 1998, *44*, 2008–2014.

[135] Giddings, M. C., Severin, J., Westphall, M., Wu, J. Z., Smith, L. M., *Genome Res.* 1998, *8*, 644–665.